

# SII Bio-property mapping system with functional nano-probes

The functional nano-probe sensor and biochip system for Bio-SPM

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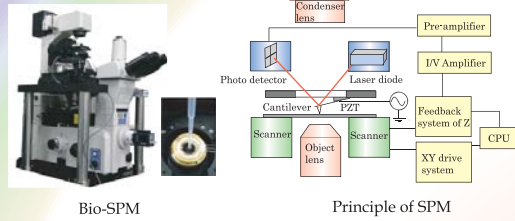
## 1. Introduction

In a research field of bio, it is important to study structures and properties of molecule order. The scanning probe microscope (SPM) has been used to the observation of biotic specimens in liquid.

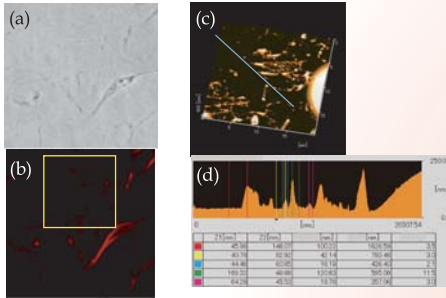
In this study, we developed Bio-SPM combined with the inverted optical microscope and SPM for the study of bio-properties. Furthermore, we realized high resolving observation of biotic specimens by developing the probe that was suitable for the measurement in liquid.

We show the new result that measured structures of lipid rafts on cultured cells by Bio-SPM.

## 2. About Bio-SPM

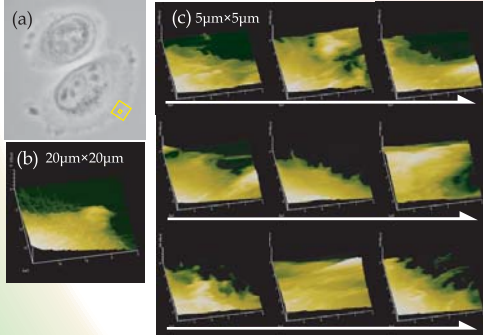


### 2-1. Simultaneous observation of SPM & OM



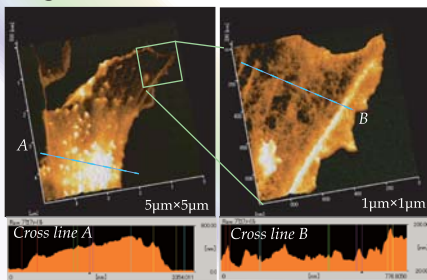
Observation of adhesion material after removing cultured cells  
(a) Phase-contrast image, (b) Fluorescence image of F-actins,  
(c) SPM image of □ in (b), (d) Cross line profile of (c)

### 2-2. Imaging of living cell



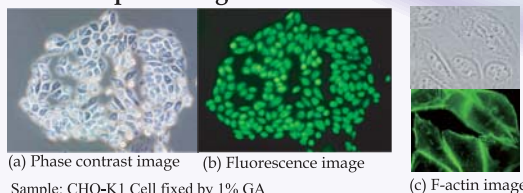
(a) Phase-contrast image of living cells, (b) SPM image of 20µm×20µm,  
(c) Moving cell image of 5µm×5µm

### 2-3. High-resolution observation of cultured cell



Sample: CHO-K1 fixed by 1% GA in PBS buffer

### 2-4. Cell patterning



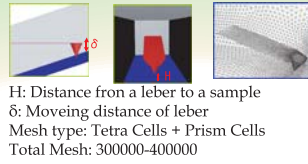
(a) Phase contrast image (b) Fluorescence image

Sample: CHO-K1 Cell fixed by 1% GA

(c) F-actin image

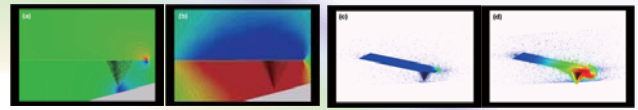
## 4. Simulation of the interaction between a Tip & a Sample

### 4-1. Simulation condition



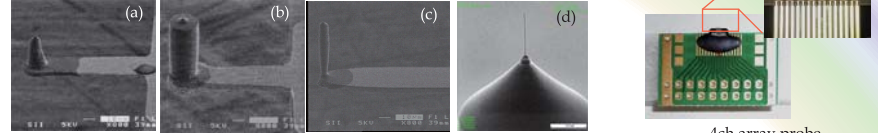
H: Distance from a lever to a sample  
δ: Moving distance of lever  
Mesh type: Tetra Cells + Prism Cells  
Total Mesh: 300000-400000

### 4-2. Simulation results



(a), (b) Pressure-contour (c), (d) Location-vector

### 4-1. Probes processed by MEMS



(a), (b) (c) Si Long Tips, (d) Carbon deposition

## 5. Observation of lipid raft domain

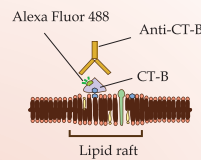
### Lipid Raft Labeling

#### Sample

Cultured CHO-K1 cells (Chinese hamster ovary cells) for 3 days.

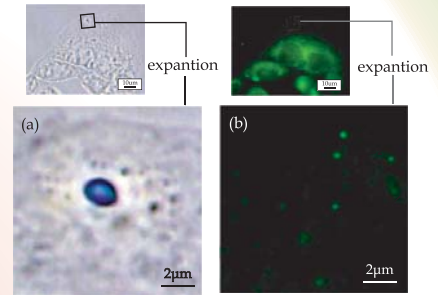
#### Materials

- Cholera toxin subunit B (recombinant) labeled with the Alexa Fluor 488
- Anti-cholera toxin subunit B antibody (anti-CT-B)
- Phosphate-buffer saline (pH 7.2)

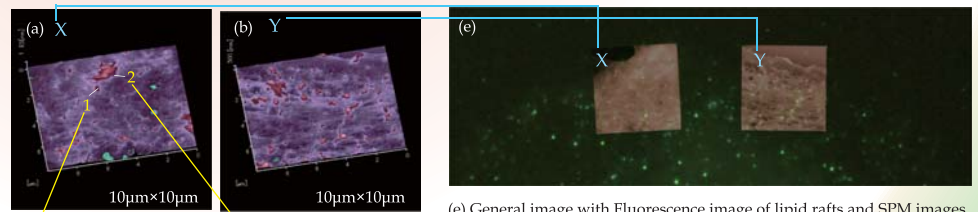


#### Labeling Protocol

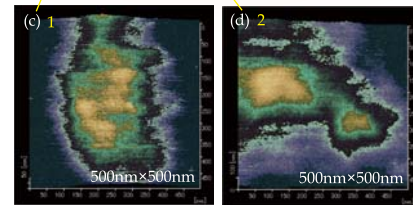
- Label the cells with the fluorescent CT-B conjugate.
  - Incubate for 20 minutes at 4°C
  - Wash the cells 3 times with chilled PBS.
- Crosslink the CT-B labeled lipid raft with the anti-CT-B antibody.
  - Incubate for 30 minutes at 4°C
  - Wash the cells 3 times with chilled PBS.
- Fix the cells.
  - Incubate in chilled PBS containing 4% formaldehyde for 15 minutes at 4°C
  - Wash the cells 3 times with chilled PBS.
- Mount and visualize the fixed cells in PBS.



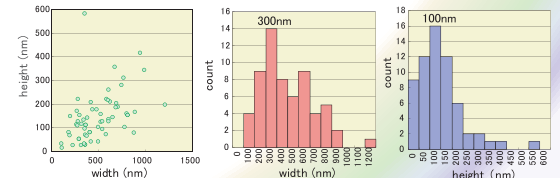
(a) Phase-contrast image  
(b) Fluorescence image of lipid rafts  
(c) General image with OM image and SPM image  
(d) 3D observation of lipid raft domains by SPM  
(e) Cross line profile



(e) General image with Fluorescence image of lipid rafts and SPM images



(a) SPM image of (e)-X (10µm×10µm )  
(b) SPM image of (e)-Y (10µm×10µm )  
(c) SPM image of (a)-1 (500nm×500nm)  
(d) SPM image of (a)-2 (500nm×500nm)



Dimension of lipid rafts

Histogram of width (500nm±231nm)

Histogram of height (145nm±102nm)

(f) Dimension of lipid rafts

## 6. Conclusion

We observe CHO cells which fluorescence labeled lipid raft domains and succeeded in observation of fluorescent images and SPM images simultaneously by Bio-SPM. We decided lipid raft domains in SPM images by fluorescent images, and measured fine structures of lipid raft domains.

In this research, we found that all the lipid raft domains formed a swelled, terrace like structures on a cell membrane with the width of 500 nm ± 231 nm and the height of 145 nm ± 102 nm. This results showed new information for biological study.

### Acknowledgments

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